New parameters for identifying subclinical atherosclerosis in patients with primary Sjögren's syndrome: a pilot study

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Abstract

Objective

We investigated sub-clinical cardiovascular involvement in primary Sjögren's syndrome (pSS) patients by means of ADMA, coronary flow reserve (CFR), intima media thickness (cIMT), pulse wave velocity (PWV) and myocardial deformation.

Methods

The study involved 22 outpatients with pSS (6 males, 16 females; mean age 60.14±7.81 years) and no documentable cardiovascular disease, and 22 age- and gender-matched controls. Dipyridamole transthoracic stress echocardiography was used to evaluate wall motion and CFR. A CFR value of <2.5 was considered a sign of impaired coronary function. We also evaluated cIMT arterial stiffness PWV and plasma ADMA levels, and made a speckle tracking echocardiography (STE) analysis.

Results

All of the patients were affected by pSS. Although within the normal range, the patients' CFR was lower than that of the controls (median 2.70; IQR 2.40–2.90 vs. 3.20; IQR 3.06–3.33; p<0.0001), whereas their ADMA levels were significantly higher (median 0.81 µM; IQR 0.79–0.85 µM vs. 0.54 µM; IQR 0.52–0.58 µM; p<0.0001). Both left and right PWV values were significantly higher in the patients than in the controls (median 8.8 m/s right and 8.9 m/s left vs. 6.86 and 6.89 m/s), whereas QIMT was substantially similar in the two groups.

Conclusion

Higher ADMA levels suggest the presence of endothelial dysfunction and sub-clinical atherosclerosis in pSS patients, even in the case of a normal CFR. This finding is supported by the PWV values, which were higher in the pSS patients. ADMA levels and PWV values may be useful markers for identifying early endothelial dysfunction in pSS patients.

Key words

Sjögren's syndrome, connective tissue diseases, atherosclerosis, arterial stiffness, speckle tracking echocardiography

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Introduction

Sjögren's syndrome (SS) is a common chronic autoimmune disease with a prevalence of 0.5–1% that is characterised by lymphocytic infiltration and progressive injury to the exocrine glands. In general, the main lesions affect the salivary and lachrymal glands and lead to dry eyes and mouth (1). The syndrome may present as a primary disease (pSS) or be associated with other autoimmune rheumatic diseases such as rheumatoid arthritis, systemic lupus erythematosus (SLE) and systemic sclerosis, which define secondary SS (2). Like many other autoimmune conditions, primary SS is triggered, by a mosaic of genetic, environmental and hormonal factors that ultimately induce immune deregulation and loss of tolerance (3). The disease spectrum ranges from sicca syndrome (4) to systemic involvement (extra-glandular manifestations), and may be complicated by the development of lymphoma; there are often also systemic features due to cutaneous, respiratory, renal, hepatic, neurological and vascular involvement often (1). Patients of all ages may be affected, although the disease generally becomes overt during the fourth and fifth decades of life with a female-to-male ratio of 9:1 (1, 2).

Although pSS predominantly affects the specific epidemiological subset of postmenopausal women, who are at higher risk of cardiovascular complications (5, 6), little is known about the clinical significance of cardiovascular (CV) disease in patients with pSS. The findings of two small case-control studies suggest that pSS may be associated with a higher frequency of cardiovascular and metabolic abnormalities. Lodde *et al*. (7) described a differentiated lipid serum profile in 46 patients with pSS, and Vaudo *et al*. (8) found a higher rate of subclinical atherosclerosis in a small group of 37 female SS patients studied by means of femoral and carotid ultrasonography. Gerli *et al*. (9) found that, although endothelium-dependent flow-mediated vasodilation was similar in 45 women with pSS and controls, endothelium-independent nitrate-mediated vasodilation was lower, which suggests that functional impairment of the arterial wall may sustain the early phases of atherosclerotic damage.

We have previously shown that plasma asymmetric dimethylarginine (ADMA) levels and coronary flow reserve (CFR) are impaired in patients with early RA (10), but it is not known whether the same is true for patients with pSS. Various methods have been used to assess endothelial dysfunction, arterial wall injury and atherosclerotic plaques, but not all of them have been validated (11). We have shown the importance of ADMA in studying early endothelial function (12), the more limited relevance of carotid intima-media thickness (cIMT) in the early stage of the disease (13, 10), the greater value of arterial stiffness parameters and pulse wave velocity (PWV) analysis (14, 15), and finally the significance of CFR in the case of suspected coronary artery disease (10, 16), and speckle tracking echocardiography (STE) in the case of subclinical cardiac involvement (17). We therefore used all of these validated methods to investigate silent cardiovascular involvement in pSS patients.

Materials and methods

Patients

The study group consisted of 22 outpatients (six males and 16 females; mean age 60.14±7.81 years), who fulfilled the American College of Rheumatology (ACR) criteria for pSS (18) but had no clinical history or signs of CV diseases, and were assessed between May and September 2012. Fourteen of the women (87.5%) were postmenopausal. None of the patients was taking hormonal replacement therapy. The control group consisted of 22 healthy volunteers matched for age, gender and other anthropometric characteristics.

In order to avoid confusion with known risk factors for atherosclerosis, the exclusion criteria were hypertension (systolic/diastolic blood pressure of ≥140/90 mmHg or the use of antihypertensive medication), hyperlipidemia (total cholesterol levels of ≥200 mg/dL, low-density lipoprotein cholesterol levels of ≥115 mg/dL or triglyceride levels of ≥150 mg/dL, or the use of lipid-lowering medication), diabetes mellitus diagnosed on the basis of the

World Health Organisation (WHO) criteria (19) or the use of antidiabetic medications, and a history of ischemic heart disease or cerebrovascular events. Further exclusion criteria were a technically poor acoustic window precluding satisfactory two-dimensional (2-D) Doppler echocardiographic imaging of the left ventricle or left anterior descending (LAD) coronary artery flow (for CFR assessment), congenital, valvular or hypertrophic cardiomyopathy, myocarditis, pericarditis, thyroid disease, severe mental retardation, and lymphoproliferative disorders.

Finally, none of the study participants had smoked cigarettes in the previous ten years.

The study protocol was approved by our local Ethics Committee and informed consent was obtained from all of the subjects before undergoing any study procedure. The study was conducted in accordance with the ethical guidelines of the 1975 Helsinki Declaration.

Cardiovascular assessment

The CV risk profile of all of the patients was assessed by means of standard electrocardiography (ECG), conventional and stress trans-thoracic echocardography with CFR measurement, and carotid ultrasonography. Trans-thoracic Doppler-derived CFR, C-INT and PWV were analysed by two independent echocardiographers not involved in the patients' care.

Arterial blood pressure and ECG were evaluated using standard procedures (20).

Standard echocardiography

The trans-thoracic echocardiographic images were recorded using a commercially available ultrasound unit (IE33, Philips Medical Systems, USA) equipped with a 1-2 MHz (S5) transducer and a 3-8 MHz broad-band high-frequency trans-thoracic transducer (S8) with second harmonics. LV diameter and wall thickness were measured using targeted two-dimensional M-mode echocardiographic traces as recommended by the American Society of Echocardiography (21). The LV was divided into 16 segments, and segmental wall motion was graded as 1 = normal, 2 = hypokinetic, 3 = akinetic, or 4 = dyskinetic; the wall motion score index was obtained by dividing the sum of the segment scores by the number of visualised segments (22).

LV mass was calculated using Devereux's formula (23). The Doppler indices of LV diastolic function were measured using standard techniques (24).

Coronary flow reserve

All of the subjects were asked to avoid xanthine-containing food and drinks for ≥24 hours before their trans-thoracic Doppler-derived CFR evaluation. With the subjects in a stable 90° left lateral recumbent position, LAD coronary flow was evaluated before and during dipyridamole infusion (0.84 mg/kg over 6 min) using a modified two-chamber view in order to identify the distal LAD artery. Coronary blood flow in the mid-distal portion of the LAD artery was measured under the guidance of colour-Doppler flow mapping, and was synchronised with the ECG. CFR was calculated off-line as the ratio between peak diastolic velocity during hyperaemia and baseline diastolic velocity (for each parameter, three cardiac cycle measurements were averaged). A CFR value of <2.5 was considered a sign of impaired coronary function. At the same time, segmental LV wall motion, ECG and symptom arousal were also evaluated, and the LV wall motion score index was calculated during stress. At the end of the protocol, all of the patients received 125–250 mg of aminophylline to counteract the effect of dipyridamole.

All of these recordings were digitally stored in order to simplify the review and measurement process.

Speckle tracking analysis

Speckle tracking analysis was performed off-line using commercially available QLAB 8 software (Philips Medical System, USA). Two-D images were obtained from the apical 4-chamber view at a high frame rate (70–80 frames/s) and three cardiac cycles were stored in cine-loop format for off-line analysis in order to assess LV end-systolic longitudinal and radial strain (ε).

The operator traced the endocardial border on an end-diastolic frame and the software automatically tracked the border on the subsequent frames. Adequate tracking can then be verified in real-time and corrected by adjusting the region of interest or manually correcting the border to ensure optimal tracking. End-systole was identified as corresponding to aortic valve closure as measured by pulsed-Doppler. The software represents myocardial deformation in the form of time-strain graphs in which it is possible to identify the different phases of the cardiac cycle as follows: a negative wave is observed during systole, which reaches its negative peak at the time of aortic valve closure and represents the maximal longitudinal myocardial shortening during contraction (Fig. 1); during diastole, the strain values progressively increase towards the original length.

The time-strain curves were obtained and analysed by two independent observers who were blinded to the clinical data. Inter-observer variability was calculated by comparing the measurements made by the two observers in 10 randomly selected subjects using the Bland-Altman method (25), and was <5% for both longitudinal and radial ε.

Carotid artery ultrasonography and stiffness measurements

Carotid artery ultrasonography was performed using a My Lab 60 (Esaote, Florence, Italy) with a 2–9 MHz LA532E linear array transducer equipped with RF-QIMT and RF-QAS software that complies with the Mannheim Consensus (26). The patients were scanned in a supine position with the neck extended and chin turned contralaterally to the examined side in order to evaluate their carotid arteries both transversely and longitudinally. The IMT of the common carotid artery (CCA) was measured 1 cm distal to the carotid bifurcation in the posterior wall of both the right and left carotid arteries. IMT was defined as the distance between the leading edges of the lumen interfaces and the media/adventitia interface of the far wall (27, 28), and recorded as the average of three measurements.

Moreover, in order to assess stiffness, the right and left CCAs were examined

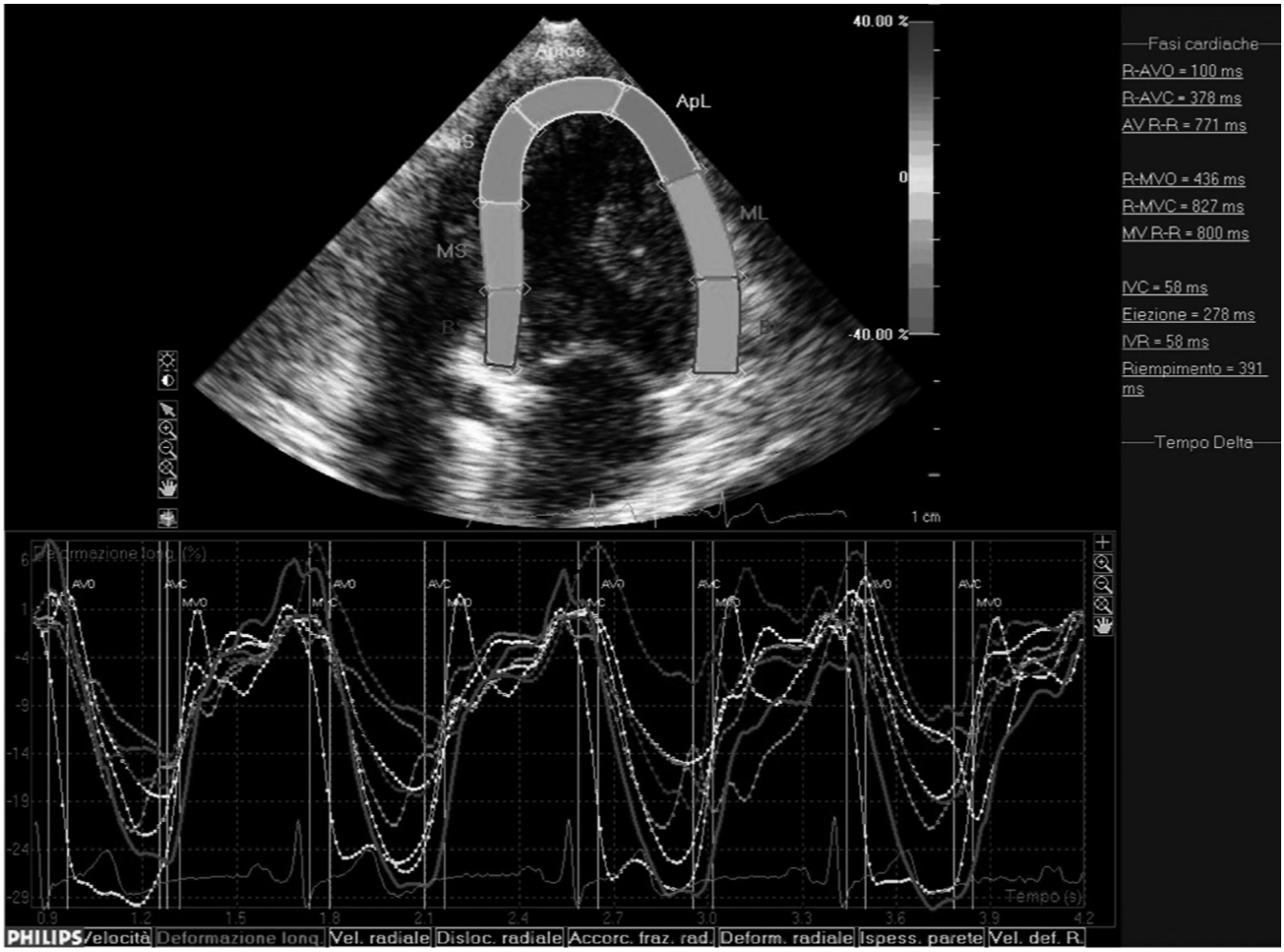

Fig. 1. Left ventricular longitudinal strain from the apical 4-chamber view in a primary SS patient: the time-strain curves show a negative end-systolic strain representing myocardial shortening during systole. End-systole was identified on the basis of aortic valve closure (AVC). During systole, the negative wave reached its negative peak upon AVC thus representing maximal longitudinal myocardial shortening during contraction; during diastole, the myocardium progressively reached its original length.

about 1 cm proximal to the bulb region. Time-related pressure waveforms were obtained from the systolic and diastolic changes in arterial diameter after calibrating blood pressure measured in the right upper arm using a cuff-type manometer.

PWV, a basic parameter of vascular stiffness, is usually based on 'two point' measurements and calculated as the time it takes the wave to travel a known distance; however, there are some methods that allow PWV to be derived from a "one point" measurement. We used radio frequency data processing of linear amplitude and phase information, which allows the automatic, highly accurate, non-invasive real-time determination of blood vessel wall diameter, the change in diameter (distension), and wall thickness, on the basis of which it is possible to calculate stiffness biomarkers such as local PWV and QIMT. These parameters were normalised to local blood pressure by rescaling the local distension waveform to brachial pressure (29).

Laboratory analyses

The pSS-related laboratory variables of erythrocyte sedimentation rate (ESR), white blood cell and platelet counts, and C-reactive protein (CRP) levels were measured using routine methods.

IgM RF was measured by means of immunonephelometry using the quantitative N Latex RF system (Dade Behring, Marburg, Germany). RF titres of >15 IU/ml were considered positive.

Anti-nuclear autoantibody levels were determined by means of indirect immunofluorescence using HEp2 cells. Antibodies against extractable nuclear antigens (ENAs) including SSA, SSB were detected by means of enzyme-linked immunosorbent assays (ELISAs).

Total serum cholesterol, triglyceride and high-density lipoprotein cholesterol levels were determined using an autoanalyser, and LDL cholesterol was calculated by means of Friedewald's formula.

Other standard clinical laboratory tests were performed under fasting conditions on the same day as the main evaluations. Mean glomerular filtration rate (GFR) was calculated using the Cockroft-Gault formula (30).

Asymmetric dimethylarginine (ADMA)

ADMA is a marker of atherosclerosis and a predictor of CV risk. Increased

plasma ADMA levels have been observed in patients with conditions associated with enhanced atherosclerosis (31), and it is known that chronic inflammation and endothelial dysfunction, which is characterised by a reduction in the bioavailability of nitric oxide (NO) (31) as a result of a NO synthase blockade that seems to be the *primum movens* of the atherosclerotic process, are important factors in the development of atherosclerotic plaque (32). ADMA is the main endogenous inhibitor of all three NO synthases, which suggests that it is involved in endothelial dysfunction and atherosclerosis (33).

Plasma ADMA levels were determined using high-performance liquid chromatography (HPLC) as described by Teerlink *et al*. (34), with minor modifications. Linearity was assessed in the range of 0.1-20 μM, and the mean correlation coefficient was >0.99. The ADMA limit of quantitation (LOQ) was 0.01 μM. Analytical recovery was 98%, and the interassay coefficient of variation was greater than 3%.

Statistical analysis

Continuous variables are expressed as mean values and standard deviations or as median values and interquartile ranges (IQR) according to variable skewness. Non-continous variables were described as percentages. The data were analysed using SAS statistical software, version 9.2. All of the tests were two-tailed, and probability (p) values of <0.05 were considered statistically significant.

Results

Table I shows the characteristics of the pSS patients and healthy controls.

All of the patients were affected by diffuse pSS, but none had any CV signs or symptoms. The majority of the patients were being treated with hydroxychloroquine (HCQ) 400 mg/day, six with azathioprine (AZA) at a mean dose of 150 mg/day (range 50-200 mg), and four with methotrexate (MTX). Four patients were receiving corticosteroids 2.5 mg/day, three 5 mg/day due to joints involvement relapse for 3-5 months, and three with nifedipine 30 mg/day because of cold-related Raynaud's phenomena. None of the of pSS patients suffered from pulmonary involvement such as interstitial lung disease or any other complication.

All of the pSS patients were ANA and RF and SSA or SSB positive and had significantly higher CRP and ESR values than the healthy controls (p<0.001 for both); however, there were no significant differences in heart rate, arterial blood pressure, age or body mass index (BMI) (Table I).

The patients' mean age, EF and E/A ratios were respectively 60.14±7.81 years, 59.77%±6.26%, and 0.92±0.23, and were not significantly different from those of the controls (Table II).

Table I. Characteristics of SS patients and controls.

	SS patients (n=22)	Controls (n=22)	p-value
No. of females (%)	16 (72.7%)	16 (72.7%)	NS
Age (years)	60.14 ± 7.81	59.25 ± 2.08	NS
BMI (kg/m^2)	23.86 ± 5.02	23.69 ± 1.12	NS
Systolic blood pressure (mmHg)	126.04 ± 17.11	125.61 ± 12.48	NS
Diastolic blood pressure (mmHg)	81.93 ± 5.95	80.45 ± 8.25	NS
Heart rate (beats/min)	65.05 ± 10.27	68.33 ± 12.69	NS
CRP (mg/dl)	7.36 ± 2.08	0.41 ± 0.09	<0.0001
ESR (mm/h)	23.33 ± 12.86	4.8 ± 0.24	<0.001
Disease duration (months)	46.06 ± 8.24	–	–

Mean values ± standard deviation (SD).
BMI: body mass index; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate. NS: not significant.

Table II. Echocardiographic parameters in the SS patients and controls.

	SS patients (n=22)	Controls (n=22)	p-value
EDV	78.65 ± 22.19	80.24 ± 7.01	NS
ESV	30.05 ± 6.43	29.24 ± 4.41	NS
LVEF (%)	59.77 ± 6.26	60.46 ± 4.53	NS
LA (mm)	34.78 ± 5.87	35.37 ± 6.03	NS
E/A	0.92 ± 0.23	0.95 ± 0.24	NS
sPAP (mmHg)	27.37 ± 6.18	26.93 ± 7.15	NS
E/E'	8.97 ± 2.16	8.88 ± 3.25	NS

Mean values ± standard deviation (SD).
EDV: end-diastolic volume; ESV: end-systolic volume; LVEF: left ventricular ejection fraction; LA: left atrium; E/A: ratio between peak velocity of early diastolic mitral flow and peak velocity of late diastolic mitral flow; sPAP: systolic pulmonary arterial pressure; E/E': ratio between peak velocity of early diastolic mitral flow and peak early diastolic myocardial velocity derived from pulsed-wave Doppler tissue; NS: not significant.

Table III. Other cardiovascular parameters.

Variables	Patients Median values [IQR]	Controls Median values [IQR]	p-value*
ADMA (μM)	0.81 [0.79 – 0.85]	0.54 [0.52 – 0.58]	<0.0001
CFR	2.70 [2.40 – 2.90]	3.20 [3.06 – 3.33]	<0.0001
IMT RCCA (mm)	0.60 [0.50 – 0.70]	0.53 [0.50 – 0.60]	NS
IMT LCCA (mm)	0.60 [0.60 – 0.70]	0.60 [0.50 – 0.60]	NS
PWV right (m/s)	8.80 [7.26 – 10.32]	6.86 [6.66 – 7.10]	<0.0001
PWV left (m/s)	8.90 [8.00 – 10.00]	6.89 [6.30 – 7.10]	<0.0001
Long. ε 4c (%)	15.28 [12.30 – 16.20]	19.80 [19.30 – 20.40]	<0.0001
Radial ε SAX (%)	26.00 [24.26 – 31.90]	31.50 [28.30 – 34.50]	0.02

IQR: interquartile range. ADMA: asymmetric dimethylarginine; CFR: coronary flow reserve; IMT: intima-media thickness; RCCA: right common carotid intima-media thickness; LCCA: left common carotid intima-media thickness; PWV: pulse wave velocity; ε: strain; Long. ε 4c: longitudinal strain deformation in apical 4-chamber view (expressed as negative values); Radial ε SAX: radial strain deformation in short axis view (expressed as negative values). *Mann-Whitney test. NS: not significant.

Although within the normal range, their CFR was lower than that of the controls (median 2.70, IQR 2.40–2.90 *vs*. 3.20, IQR 3.06–3.33; $p<0.0001$), whereas their ADMA levels were significantly higher (median 0.81 μM, IQR 0.79–0.85 μM *vs*. 0.54 μM, IQR 0.52–0.58 μM; $p<0.0001$) (Table III) (Fig. 2).

Both right and left PWV values were significantly higher in the pSS patients (Table III), as were both right and left cIMT values, but these differences were not statistically significant (Table III) (Fig. 2). Speckle tracking analysis was significantly different between the two groups, with longitudinal strain deformation in the apical four chambers view (Long. ε 4c) and radial strain deformation in short axis view (radial ε SAX) being significantly less in the pSS patients.

Moreover, there was no relationship between RF, anti-Ro and anti-La antibodies or inflammatory markers and the parameters of subclinical atherosclerosis.

Discussion

Cardiac involvement in primary SS was described in the early 1970s by Shearn in his monograph, but the reported cases corresponded to secondary SS and were usually associated with SLE (35-40).

Our patient cohort showed impaired CFR and increased ADMA levels. It has recently been demonstrated that CFR is a highly sensitive (>90%) diagnostic marker of CAD, and that a CFR of <2 accurately predicts the presence of severe coronary stenosis (*i.e.* >70% coronary narrowing) (41). Higher ADMA levels suggest the presence of endothelial dysfunction and sub-clinical atherosclerosis in primary SS patients, even in the case of normal CFR, as we have previously demonstrated in patients with other systemic rheumatic diseases such as rheumatoid arthritis (RA) (10), psoriatic arthritis (PsA) and systemic sclerosis (SSc) (42, 15). High ADMA levels in patients with systemic rheumatic diseases may be due to a number of mechanisms, including increased endothelial cell turnover and the ensuing release of free ADMA during protein catabolism, which suggests that various mechanisms may also be involved in primary SS.

The subclinical CV involvement in our pSS did not seem to be related to the conditions usually associated with high plasma ADMA levels, which we used as exclusion criteria. One recent paper has shown that pSS patients have a higher incidence of diabetes mellitus and hypertriglyceridaemia and a lower incidence of hypertension and smoking than controls (43). Moreover, the patients who had received corticosteroids were more frequently affected by hypertension, diabetes mellitus and hypertriglyceridaemia. As there was no significant difference in the presence of any of these risk factors for atherosclerosis between our pSS patients, ADMA may be an independent indicator of subclinical atherosclerosis in pSS. Furthermore, the seven patients treated with low-dose corticosteroids because of relapsing joint involvement were only treated for short periods.

We found that both right and left PWV values and the β stiffness index were significantly higher in the pSS patients than controls. This is in line with the data of Gerli *et al*. (10) who found that, although endothelium-dependent FMV values were similar in 45 women with pSS and controls, endothelium-independent nitrate-mediated vasodilation (NMV) values were lower in the patients, thus suggesting that functional impairment of the arterial wall may sustain the early phases of atherosclerotic damage in pSS.

Patients with pSS seem to have higher common carotid artery IMT values than healthy controls, although the difference was not statistically significant in our case. This may have been due to the relatively small number of patients, but it could also indicate that the functional parameter CFR is a more sensitive marker of subclinical atherosclerosis than cIMT, as we have previously found in other systemic autoimmune diseases (10, 15, 37).

Most of the patients were taking HCQ. As the use of antimalarials can decrease total and LDL cholesterol and triglyceride concentrations, and decrease the risk of thrombosis in patients with systemic lupus erythematous (SLE) (44), the administration of HCQ may have led to less endothelial dysfunction. However, the majority of the patients (87.5%) were postmenopausal women and none of them were taking hormonal replacement therapy, thus making them a specific epidemiological subset with higher risk of cardiovascular complications (5, 6).

An echocardiographic examination study of 64 pSS patients showed an echogenic pericardium in 21 patients who had previous symptom-free pericarditis, a normal left ventricular (LV) systolic function, and impaired diastolic function in 42 (45). LV diastolic dysfunction is a major cause of cardiac morbidity and seems to be one of the earliest detectable abnormalities in disorders such as hypertension, diabetes mellitus, and ischemic heart disease (46). Moreover, LV diastolic dysfunction has also been seen in patients with long-standing RA without CD events or traditional cardiovascular risk factors (47). Our pSS patients seemed to have similar LV systolic and diastolic function to that of the healthy controls as evaluated by means of standard echocardiography. However, we have found that LV radial and longitudinal ε are impaired in pSS patients without any clinical or STE evidence of CV involvement. On the basis of these findings and the sensitivity of radial ε in detecting subclinical LV systolic dysfunction (17), it can be hypothesised that chronic inflammation and early impairment of the coronary microcirculation can affect myocardial ε in pSS patients. Moreover, these data confirm the importance of developing more precise non-invasive tools to improve sensitivity in detecting premorbid cardiac diseases in all patients with systematic rheumatic diseases.

In conclusion, various methods indicate that patients with diffuse pSS seem to have subclinical CV involvement. The higher ADMA levels in primary SS patients suggest the presence of endothelial dysfunction and sub-clinical atherosclerosis, even in the case of a normal CFR. Our preliminary data indicate that ADMA may be a useful marker for identifying early endothelial dysfunction in such patients. Moreover, the LV myocardial radial and longitudinal ε measured by means of STE

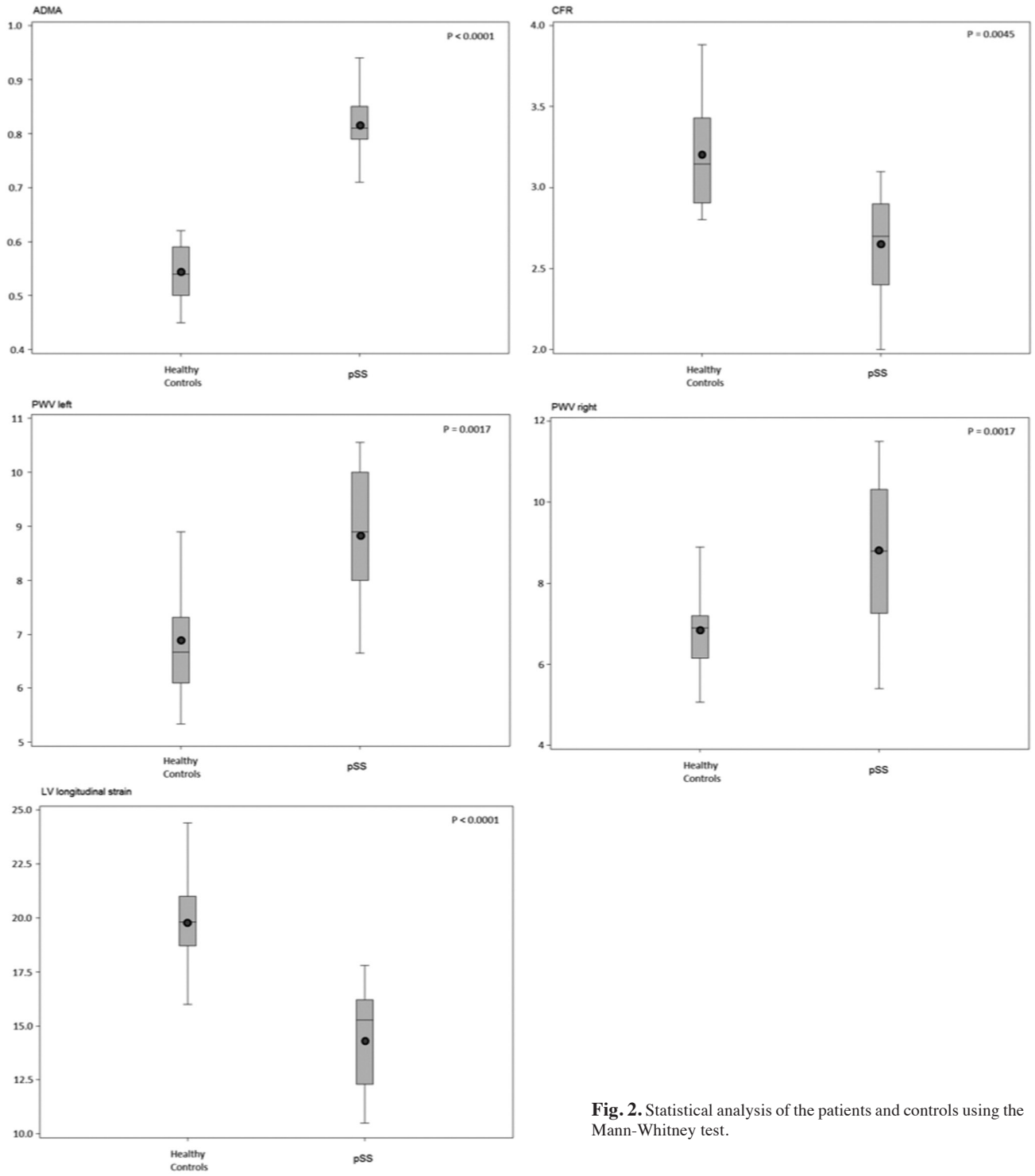

Fig. 2. Statistical analysis of the patients and controls using the Mann-Whitney test.

were impaired in our pSS patients in the absence of any clinical evidence of CV disease and when traditional echocardiographic evaluations were still negative, thus suggesting a myocardial alteration. However, further studies are required to define more precise methods for assessing and determing CV disease in patients with pSS.

Study limitations

The main limitation of this study was the small number of patients, but the size of our cohort was similar to those of other similar studies and the number of the tests used was higher.

Authors' contributions

F. Atzeni conceived the idea for this study and, together with M. Turiel, L. Gianturco and P. Sarzi-Puttini, participated in the design and coordination of the study, and helped to draft the manuscript. C. Ricci performed the statistical analysis. All the authors participated in the patients selection and examination, and all read and approved the final manuscript.

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